# Characterization, Genetic Variation, and Combining Ability of Maize Traits Relevant to the Production of Cellulosic Ethanol

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#### **ABSTRACT**

Maize (Zea mays L.) stover has been identified as an important feedstock for the production of cellulosic ethanol. Our objectives were to measure hybrid effect and combining ability patterns of traits related to cellulosic ethanol production, determine if germplasm and mutations used for silage production would also be beneficial for feedstock production, and examine relationships between traits that are relevant to selective breeding. We evaluated grain hybrids, germplasm bred for silage production, brown-midrib hybrids, and a leafy hybrid. Yield and composition traits were measured in four environments. There was a 53% difference in stover yield between commercial grain hybrids that were equivalent for other productionrelated traits. Silage germplasm may be useful for increasing stover yield and reducing lignin concentration. We found much more variation among hybrids than either in vitro ruminal fermentability or polysaccharide concentration. Correlations between traits were mostly favorable or nonexistent. Our results suggest that utilizing standing genetic variation of maize in breeding programs could substantially increase the amount of biofuels produced from stover per unit area of land.

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**Abbreviations:** ADF, acid detergent fiber; ADL, acid detergent lignin; COI, crossover interaction; DM, dry matter; GCA, general combining ability; IVR, in vitro ruminal fermentation; NDF, neutral detergent fiber; NIRS, near infrared spectroscopy; SCA, specific combining ability; SSF, simultaneous saccharification and fermentation; TEP, theoretical ethanol potential; TE yield, theoretical ethanol yield.

 $\mathbf{M}_{\text{Source}}$  (Zea mays L.) stover has been identified as a potential source of feedstock for the production of cellulosic ethanol because of its abundance and proximity to existing grain ethanol plants (Perlack et al., 2005; Schubert, 2006). Moreover, a recent life-cycle analysis concluded that crop residues, such as maize stover, would provide greater reductions in greenhouse gas emissions than dedicated energy crops because a change in land use is not necessary (Searchinger et al., 2008). Facilities using maize stover as an ethanol feedstock may be in operation soon, and the construction of additional plants—termed biorefineries—is likely given rising oil prices and enhanced profitability through advances in processing technology, enzymes, fermentation organisms, and thermochemical methods for synthesizing other types of biofuels (Roman-Leshkov et al., 2007; Service, 2007). One cellulosic ethanol facility being added to an existing maize grain ethanol plant in Emmetsburg, IA (POET, Sioux Fall, SD) will utilize maize cobs as a feedstock in the near term (Service, 2007).

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Cobs may be the most attractive feedstock because of their higher bulk density, which makes their storage and transportation more economical.

The process currently envisioned for production of cellulosic ethanol involves thermochemical pretreatment of the feedstock followed by enzymatic hydrolysis and fermentation (Himmel, 2007). Enzymatic hydrolysis, which releases monosaccharides from the complex, cell wall polysaccharides, is combined with the fermentation step in a process called simultaneous saccharification and fermentation (SSF). Fermentation organisms have been modified to ferment both pentose and hexose sugars, greatly increasing the amount of ethanol that can be produced when compared to natural fermenting organisms (Yanase et al., 2007). Various other processes for generating other types of biofuels from cellulosic sources are being investigated and could be more widely used in the future because of their advantages over ethanol (Tollefson, 2008).

Regardless of the exact processing technology used or type of biofuel produced, quality of cellulosic feedstocks will mostly be a function of total polysaccharide concentration and the convertibility of those polysaccharides (Dien et al., 2006). Theoretical ethanol potential (TEP) is the maximum amount of ethanol that can be produced per unit mass of feedstock and is calculated from concentrations of the different structural carbohydrates (USDOE, 2006). Convertibility is the percentage of those carbohydrates converted into ethanol using feasible pretreatment and hydrolysis schemes. Increasing the convertibility of maize stover through breeding could significantly lower processing costs and thus enhance the economics of ethanol from maize stover or cobs (Lynd et al., 2008). However, if advances in processing technologies make 100% conversion of structural carbohydrates feasible, TEP will be the most salient factor in feedstock quality.

Variation among hybrids for stover yield and quality could be exploited by hybrid recommendation and breeding to increase the amount of cellulosic ethanol produced from an area of land. Results pertaining to this have not been published, but the use of maize stover for liquid fuels production is analogous to using forages for ruminant livestock nutrition.

A large amount of intraspecific genetic variation for forage yield and quality exists, and the development of improved cultivars through selection can significantly impact the economics of milk and meat production (Burton, 1982; Casler and Vogel, 1999). Choice of maize hybrid has a significant effect on the potential volume of milk produced from an area of land when whole plant forage is fed to dairy cattle in the form of silage (Lauer et al., 2001; Frey et al., 2004). This has led to breeding programs devoted to improving maize as a forage crop and the release of silage-specific hybrids. In addition to endogenous variation, important sources of genetic

variation include brown-midrib (bm1, bm2, bm3, bm4) and leafy mutants (Lfy1). Hybrids carrying the Lfy1 allele typically have at least four additional leaves above the ear and thus produce more stover (Shaver, 1983). The recessive bm alleles each confer a lesion in the lignin biosynthetic pathway, which can result in highly digestible stover (Coors and Lauer, 2001). Hybrids carrying the bm and Lfy1 alleles have been useful for silage production and may be beneficial for feedstock production as well.

Our objectives were (i) to measure the effect of hybrid choice on feedstock yield (both total stover and cob) and compositional properties related to quality, (ii) to determine if germplasm or mutations used for silage production have characteristics desirable for feedstock production, (iii) to measure combining ability patterns of traits potentially relevant to feedstock production, and (iv) to evaluate relationships between these traits that may be of consequence to selective breeding and determine if the easy and inexpensive detergent fiber analysis system commonly used in the field of ruminant nutrition is adequate for predicting relative values of theoretical ethanol potential. To accomplish this, we evaluated stover yield, cob yield, TEP, detergent fiber concentration, in vitro ruminal digestibility, and other production- and composition-related traits on a set of hybrids including germplasm bred for grain and silage production as well as brown-midrib and leafy types. A subset of the hybrids was produced using a factorial mating design to study general combining ability (GCA) and specific combining ability (SCA) effects for all measured traits. Evaluations were performed in four environments, which allowed us to study the importance of GCA effect × environment interaction to determine if extensive multiple environment testing is needed to select parents that perform well over a broad range of environments. The results presented herein document the impact of hybrid choice on cellulosic ethanol production from maize and will help begin the process of designing breeding programs to improve maize for this purpose.

### **MATERIALS AND METHODS**

## Germplasm

A set of 49 cultivars was selected to be diverse for grain yield, forage yield, and forage quality. Eight conventional hybrids bred for grain production were included (hereafter referred to as *grain hybrids*). Four grain hybrids were obtained from Dekalb Genetics (DK5143) and Pioneer Hi-Bred (P34B23, P34M93, P34N44). The other four grain hybrids were produced by crossing inbred lines from Holden's Foundation Seeds in the University of Wisconsin–Madison breeding nursery (HC33 × LH287, LH198 × LH279, LH227 × LH279, LH244 × LH287). Twentynine entries were produced by a factorial mating design. Parents included inbred lines bred for silage production at the University of Wisconsin (W601S, W602S, W603S, W604S, W605S) and tester inbred lines from Holden's Foundation Seeds (HC33, LH198, LH227, LH244, LH332) and Thurston's Genetics (TR7245).

All possible crosses were made between these two sets of inbred lines, with the exception of W605S × LH227. These entries will be referred to as silage hybrids hereafter. A balanced, 25-entry subset of the silage hybrids (W601S-W605S each crossed to HC33, LH198, LH244, and LH332) was used in the combining ability analysis. The Wisconsin Quality Synthetic population in its third cycle of recurrent selection (WQS C3) was evaluated on a per se basis and in hybrid combination with four commercial tester inbreds (HC33, LH198, LH332, TR7245). WQS C3 has undergone recurrent selection based on testcross performance for forage yield and quality (Frey et al., 2004). A brown-midrib isogenic series including the four known maize bm alleles (bm1, bm2, bm3, bm4) in a W64A × A619 background was evaluated. Finally, two commercial hybrids marketed for silage production were included. Those included commercial brown-midrib hybrid F697 (Mycogen, Indianapolis, IN) carrying the bm3 allele and commercial hybrid N48V8 (Syngenta Seeds, Basel, Switzerland) carrying the Lfy1 allele. The recessive bm alleles each confer a lesion in the lignin biosynthetic pathway, which lowers the lignin content and increases the digestibility of the stover (Coors and Lauer, 2001).

### **Field Evaluation**

The entries were evaluated at the Madison and Arlington, WI, locations during summers 2005 and 2006. Average temperatures for both seasons were close to the historical average. Precipitation levels were below average for 2005 and average for 2006 at both locations. In 2005, there was a lack of rainfall before anthesis, and plants exhibited drought stress during vegetative stages. Soil type at both locations is Plano silt loam (fine-silty, mixed, superactive, mesic Typic Arguidoll). Field plots were arranged in a randomized complete block design with three replications. Field plots consisted of two rows (6.08 m long.

in a randomized complete block design with three replications. Field plots consisted of two rows (6.08 m long, 0.76 m apart) planted to a density of 79,040 plants ha<sup>-1</sup>. A brief description of each trait measured is given in Table 1. Grain yield was measured by harvesting ears of 10 consecutive center plants of each row. Grain weights per plot were taken after ears were dried and shelled. Moisture was measured after shelling, and grain weights were adjusted to 15.5 g kg<sup>-1</sup> moisture and converted to megagrams per hectare at 79,040 plants ha<sup>-1</sup>. Cobs were saved, dried in a forage dryer for 1 wk, and weighed to determine cob yield per plot. Stover yield (minus cobs) for Madison and Arlington in 2005 and Arlington in 2006 were measured by cutting 10 plants from the center of each row approximately 15 cm above ground level. Ears were removed, the remaining plant parts were fed through a forage chopper, and weights were recorded. In the Madison-2006 environment, ears were removed from all plants, leaving the husk attached to the stalk, and whole plots were machine harvested. Stand counts were taken before harvest, and stover yields (minus cob) were adjusted to a constant planting density of 79,040 plants ha<sup>-1</sup>. Stover yield is reported as the sum of cob, leaf, stalk, and husk dry matter (DM) yield. Samples were obtained from each plot and dried at 55°C for 7 d for DM determination and compositional analysis. Days to midpollen was measured at the Madison location only and was determined when half the plants in a plot were shedding pollen.

### **Compositional Analysis**

Dried stover (minus cob) and cob samples were ground with a hammer mill to pass a 1-mm screen and subsequently scanned with a NIRSystems 6500 near infrared reflectance spectrophotometer. We used the Stover9 near infrared spectroscopy (NIRS) calibration (Hames et al., 2003) of the National Renewable Energy Laboratory (Golden, CO) to obtain a detailed compositional profile of all stover (minus cob) samples. The Stover9 calibration is a broad calibration for prediction of many chemical constituents of maize stover, including all structural carbohydrates (glucan, xylan, galactan, arabinan, mannan) and lignin (sum of acid soluble and insoluble lignin). Samples selected for calibration were analyzed for structural carbohydrate composition using high performance liquid chromatography methods derived from the Uppsala method (Theander et al., 1995). The lignin and structural carbohydrate protocol can be found at http://www.nrel.gov/biomass/analytical\_procedures.html (verified 5 Dec. 2008). Stover9 calibration statistics are displayed in Table 2. Four samples were considered spectral outliers and were excluded from the dataset. Pure cob samples are outside the range of the Stover9 calibration and were not analyzed with this tool.

Cellulose, hemicellulose, and acid detergent lignin (ADL) were also measured using the detergent fiber system (Van Soest, 1967). These measurements were taken in addition to the Stover9 predictions to determine if the rapid and less costly detergent fiber analysis can substitute for the Stover9 compositional analysis, which is based on the costly Uppsala dietary fiber analysis, for estimating biofeedstock composition and quality. Also, we used detergent fiber data to estimate cob glucan and xylan +

Table 1. Description of traits measured in the field and on samples of the maize entries obtained from field plots.

Trait	Description
Grain yield, Mg ha <sup>-1</sup>	Grain yield adjusted to 155 g kg <sup>-1</sup> moisture
Stover yield, Mg ha <sup>-1</sup>	Sum of cob, leaf, stalk, and husk dry matter yield
Cob yield, Mg ha <sup>-1</sup>	Cob dry matter yield
Stover dry matter, %	Leaf, stalk, and husk dry matter percentage at harvest
Days to midpollen, d	Days until 50% of plants in a plot were shedding pollen
Stover TEP,† L Mg <sup>-1</sup>	TEP of leaf, stalk, and husk fraction.
Cob TEP, L Mg <sup>-1</sup>	TEP of cob fraction.
Stover TE <sup>†</sup> yield, L ha <sup>-1</sup>	Theoretical ethanol yield on a unit area basis. Calculated by multiplying stover TEP by stover yield. Assumes cob fraction has TEP equal to leaf, stalk, and husk fraction.
Cob TE yield, L ha <sup>-1</sup>	Theoretical ethanol yield of cobs on unit area basis. Calculated by multiplying cob TEP by cob yield.
Glucan, g kg <sup>-1</sup>	Predicted by Stover9 NIRS <sup>†</sup> calibration.
Xylan, g kg <sup>-1</sup>	Predicted by Stover9 NIRS calibration.
Lignin, g kg <sup>-1</sup>	Predicted by Stover9 NIRS calibration. Sum of acid soluble and insoluble lignin.
Stover hemicellulose, g kg <sup>-1</sup>	NDF-ADF <sup>†</sup>
Stover cellulose, g kg <sup>-1</sup>	ADF-72% H <sub>2</sub> SO <sub>4</sub> residue
Stover ADL,† g kg-1	72% H <sub>2</sub> SO <sub>4</sub> residue-ash
Cob hemicellulose, g kg <sup>-1</sup>	NDF-ADF
Cob cellulose, g kg <sup>-1</sup>	ADF-72% H <sub>2</sub> SO <sub>4</sub> residue
Cob ADL, g kg <sup>-1</sup>	72% $\rm H_2SO_4$ residue-ash

<sup>†</sup>TEP, theoretical ethanol potential; TE, theoretical ethanol; NIRS, near infrared spectroscopy; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.

Table 2. Stover9 calibration statistics for glucan, xylan, and lignin. Stover9 is a global near infrared spectroscopy calibration used to predict the composition of maize stover and was developed at the National Renewable Energy Laboratory in Golden, CO.

Constituent	Equation	Ν	Min.†	Max.†	$R^2$	SECV <sup>‡</sup>
Glucan	1-4-4-1	77	25.7	41.4	0.68	2.0
Xylan	1-4-4-1	77	11.2	30.8	0.81	1.5
Lignin	1-4-4-1	74	6.8	25.1	0.85	1.5

<sup>†</sup>Minimum and maximum sample concentrations in calibration set.

arabinan content after adjustments were made based on the stover data (described below). Calibration sets for wet-lab analysis were selected for each year and sample-type combination using the SELECT procedure (Shenk and Westerhaus, 1991) with a standardized H value of 1.0. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and ADL were determined sequentially using the Ankom (Ankom Technology Corporation, Fairport, NY) Filter Bag method. Cellulose was determined as the percentage of tissue lost between the acid detergent step and extraction with 72% sulfuric acid. Hemicellulose was determined as the percentage of tissue lost between the neutral detergent step and acid detergent step. Acid detergent lignin is acid insoluble residue minus ash (Van Soest, 1994). Modified partial least squares calibrations were developed with the NIRS software of Infrasoft International v. 3.11 (1995). Equations used to predict constituent concentrations of all samples were selected on the basis of a high  $R^2$  and low standard error of cross-validation (SECV). Statistics for NIRS calibration were good, with  $R^2$  values > 0.80 and SECV values < 3.2% of the constituent mean with the exception of ADL (Table 3).

Structural carbohydrate concentrations were used in the National Renewable Energy Laboratory's theoretical ethanol

Table 3. Near infrared spectroscopy (NIRS) statistics for maize stover and cob calibrations of cellulose, hemicellulose, and acid detergent lignin (ADL).

	NIRS statistics											
Constituent	Equation	Mean	N	R <sup>2</sup>	SEC†	SECV†						
	Stover 2005											
Hemicellulose	1-4-4-1	33.3	118	0.92	0.68	0.78						
Cellulose	1-4-4-1	36.2	112	0.93	1.01	1.12						
ADL	1-4-4-1	4.47	109	0.59	0.90	1.06						
	Stover 2006											
Hemicellulose	1-2-2-1	30.7	118	0.84	0.66	0.78						
Cellulose	1-4-4-1	36.1	105	0.95	0.70	0.80						
ADL	2-2-2-1	4.94	109	0.62	0.82	1.01						
			Cob 2	005								
Hemicellulose	1-4-4-1	43.3	114	0.82	0.54	0.69						
Cellulose	1-4-4-1	38.5	117	0.92	0.60	0.75						
ADL	1-4-4-1	6.77	120	0.79	0.78	1.04						
			Cob 2	006								
Hemicellulose	1-4-4-1	41.4	79	0.85	0.82	1.01						
Cellulose	1-4-4-1	35.7	77	0.96	0.69	0.86						
ADL	1-4-4-1	4.3	77	0.65	0.60	0.68						

†SEC, standard error of calibration; SECV, standard error of cross validation.

yield calculator (USDOE, 2006) to calculate TEP (L Mg<sup>-1</sup>) for each sample. Theoretical ethanol potential assumes that 100% of structural carbohydrates is converted to ethanol. The theoretical ethanol yield calculator weights hexose and pentose sugars differently because of differences in monosaccharide density when in polysaccharide form (USDOE, 2006). To estimate the TEP of cobs, we assumed the detergent cellulose fraction to consist solely of glucan and the detergent hemicellulose fraction to consist solely of pentoses (hemicellulose is mainly composed of xylan [Saha, 2003]). According to our stover data, using detergent cellulose in place of glucan and detergent hemicellulose in place of xylan + arabinan would upwardly bias TEP. To correct for this, we multiplied cob cellulose by 0.85 and cob hemicellulose by 0.64. These values were used because stover glucan concentration was 85% of stover cellulose concentration and stover pentose (xylan + arabinan) concentration was 64% of stover hemicellulose concentration. Similar differences in cell wall content between the Van Soest detergent method and the Uppsala method were found by Theander and Westerlund (1993). The adjusted cob cellulose and hemicellulose concentrations were converted to cob TEP with the theoretical ethanol yield calculator (USDOE, 2006).

We used a in vitro ruminal fermentation (IVR) assay as a surrogate measure of actual ethanol yield that might be achieved when an SSF-related process is used (Weimer et al., 2005). Stover (minus cob) samples from 2005 were measured in duplicate with this assay. A detailed description of this method is given in Weimer et al. (2005). Samples were weighed into 60-mL serum bottles (~100 mg). After addition of reducing buffer and inoculation with rumen fluid, gas accumulation at 24 h was determined with a digital pressure gauge. Gas accumulation is reported as milliliters of gas per gram of dry biomass. In vitro ruminal fermentation was compared to TEP with simple linear regression of entry means calculated using 2005 data only.

### **Statistical Analysis**

Data were fit to a general linear model that included entry (fixed), environment (random), entry × environment interaction (random), and replication nested within environment (random). Mean squares were obtained from the MIXED procedure (Type 3) of SAS and adjusted means were calculated where missing observations occurred. Mean squares for the entry × environment interaction were used as the error term in determining the significance of the entry source of variation and in calculating the least significant difference (LSD) among entry means. Coefficient of genetic variation (CV<sub>G</sub>) was computed by dividing the standard deviation of entry means by the experiment mean for each trait. When the complete set of entries was considered, the distribution of trait means often significantly deviated from normality due mostly to the brown-midrib and leafy mutants, and the extreme compositional attributes of the WQS C3 germplasm. Therefore, we used only the 29 silage hybrids for the correlation analysis to fulfill distribution assumptions of the correlation coefficient and avoid bias in correlation estimates. Also, using only hybrids produced with a mating structure may provide correlation estimates closer to true genetic correlations. Entry means over environments and replications were used for the calculation of phenotypic correlations.

<sup>‡</sup>SECV, standard error of cross validation.

### **Combining Ability Analysis**

The 25 hybrids produced by crossing five silage inbreds with five tester inbreds in a factorial mating design were analyzed separately, and hybrid and hybrid × environment interaction sums of squares were partitioned into sources of variation according to the following model:

$$\begin{split} Y_{ijkl} &= \mu + G_i + G_j + S_{ij} + E_l + (GE)_{il} \\ &+ (GE)_{il} + (SE)_{ijl} + R_k(E_l) + e_{ijkl} \end{split}$$

where i = 1, 2, 3, 4, 5; j = 1, 2, 3, 4, 5; k = 1, 2, 3; l = 1, 2, 3, 4;  $Y_{iibl}$  denotes the value of the hybrid of the *i*th silage inbred, the *j*th tester, in the *k*th block, and in the *l*th environment;  $\mu$  = the grand mean;  $G_i = GCA$  effect common to all hybrids of the *i*th silage inbred;  $G_j = GCA$  effect common to all hybrids of the *j*th tester inbred;  $S_{ij} = SCA$  effect common to all hybrids produced by mating the *i*th silage inbred with the *j*th tester inbred;  $E_i$  = average effect of the *l*th environment;  $(GE)_{il}$  and  $(GE)_{il}$  = interaction between GCA effects and environments;  $(SE)_{ijl}$  = interaction between SCA effect and environments;  $R_{i}(E_{i}) = \text{effect}$ of the kth block nested within the lth environment; and  $e_{iibl}$  = random experimental error. Hybrid, GCA, and SCA sources of variation were tested for significance by using their respective environment interaction mean squares. Error mean squares were used to test significance of interaction terms. The ratio of  $G_i$  sums of squares +  $G_i$  sums of squares to hybrid sums of squares was calculated and used to estimate the relative importance of additive and nonadditive genetic effects for each trait (Pixley and Frey, 1991).

The GCA effects for each parent were calculated as:

$$G_i = (Y_i - Y)$$

$$G_i = (Y_i - Y)$$

where  $Y_i$  is the mean of hybrids derived from silage inbred i,  $Y_j$  is the mean of hybrids derived from tester inbred j, and Y is the mean of all hybrids. If GCA sources of variation were significant in the analysis of variance, individual inbred and tester GCA effects were tested for significance by calculating two-tailed t tests, where  $t = G_i/SE_{GCA}$  or  $G_j/SE_{GCA}$ . Standard errors for GCA effects were calculated as originally described by Cox and Frey (1984) and followed by Lee et al. (2005). Where the GCA  $\times$  environment interaction source of variation was significant, we tested for crossover interactions (COIs) between all pairs of silage inbreds or tester inbreds in each pair of environments (five silage inbreds or tester inbreds and four environments = 60 tests). Statistical significance of COIs was determined using the Azzalini–Cox test following Baker (1988) with an interactionwise Type I error rate of 0.05 (Cornelius et al., 1992).

# RESULTS AND DISCUSSION Stover Yield, Cob Yield, and Grain Yield

Highly significant (P < 0.01) differences were found between entries for all traits measured. The median stover yield was 9.24 Mg ha<sup>-1</sup> and, when the brown-midrib series is excluded, the range from the lowest entry (6.97 Mg ha<sup>-1</sup>) to the highest entry (10.91 Mg ha<sup>-1</sup>) was 43% of the study median (Table 4). There was more variation among entries for grain yield and cob yield compared

with stover yield. The ranges in cob and grain yield both exceeded 80% of the study medians and the  $\mathrm{CV_G}$  for grain and cob yield were both higher than that of stover yield (Table 4). Eighteen of the 49 entries were within one LSD of the highest entry for stover yield. For grain yield and cob yield, respectively, 17 and 8 entries were within one LSD of the highest-yielding entry (Table 4). Severe lodging in 2005 at both locations and at the Arlington location in 2006 forced us to harvest plots by hand, which likely resulted in more experimental error compared to standing plots harvested by machine. Therefore, our power to detect differences in stover yield between entries may have been less than under more optimal conditions.

There were large differences in stover yield among hybrids bred for grain production, suggesting that large increases in stover yield might be achieved through hybrid screening and recommendation. For instance, LH198 × LH279 yielded 53% more stover than DK5143, while being equivalent for grain yield, stover DM, and days to midpollen (Table 4). Three of the eight grain hybrids were statistically indistinguishable in stover yield from the highest-yielding entry (W605S × LH198). Cob yield did not greatly vary among the grain hybrids. Exceptions were DK5143 and P34N44, which had lower cob yield than the other six grain hybrids (Table 4). There were no differences between the eight grain hybrids with respect to grain yield, however, and evaluating cob yield may be advantageous for breeding and recommending hybrids with greater cob yield to producers located near cob markets.

# Stover and Cob Composition and Theoretical Ethanol Yield

There was significant genetic variation for stover and cob composition, resulting in differences between hybrids for stover and cob TEP (Tables 4 and 5). Stover TEP was calculated by inserting the structural carbohydrate concentrations predicted by the Stover9 NIRS calibration into the theoretical ethanol yield calculator (USDOE, 2006). Cob TEP was also calculated by the theoretical ethanol calculator, but cellulose and hemicellulose concentrations, as determined by the detergent fiber system, were used to estimate hexose and pentose carbohydrates, respectively. Therefore, stover TEP and cob TEP cannot be directly compared. Cob TEP provides only a crude, relative estimate of how much ethanol could theoretically be produced per unit mass of cob material, and we used it to assign a practical value to the variation in cob composition between hybrids. The amount of variation among entries was less for both cob TEP and cob cellulose compared to their stover counterparts. The composition of both stover and cob did not vary nearly as much as their yield (Tables 4 and 5). As discussed above, TEP is strictly a function of structural carbohydrate concentration, and we are not certain how genotypic variation in actual ethanol yield per

Table 4. Entry means for grain yield, stover yield, cob yield, stover dry matter (DM), days to midpollen, stover theoretical ethanol potential (TEP), cob TEP, stover TE yield, and cob TE yield. Tabled values are means over three replications in each of four environments.

Entry	Grain yield	Stover yield	Cob yield	Stover DM	Days to midpollen	Stover TEP	Cob TEP	Stover TE yield <sup>†</sup>	Cob TE yield <sup>†</sup>
		—— Mg ha <sup>-1</sup> —		%	d	L M		———L h	
W64A × A619	5.04	8.31	0.96	43.5	65	353	420	2972	441
W64A × A619 bm1	2.43	7.17	0.54	38.8	67	325	407	2300	221
W64A × A619 bm2	2.27	7.00	0.45	37.9	66	309	381	2262	180
N64A × A619 bm3	3.10	6.82	0.63	38.3	66	340	412	2339	264
N64A × A619 bm4	3.61	6.11	0.64	41.0	66	339	420	2081	244
N601S × HC33	11.33	9.74	1.48	46.2	70	408	437	4008	650
N601S × LH198	10.36	9.80	1.45	42.2	72	394	440	3891	597
N601S × LH227	10.44	8.96	1.48	49.6	70	407	442	3659	655
N601S × LH244	10.92	10.27	1.49	46.2	71	406	439	4198	653
N601S × LH332	11.14	10.49	1.70	45.9	71	410	442	4341	750
N601S × TR7245	10.08	9.23	1.27	46.3	70	410	446	3793	570
V602S × HC33	9.97	8.57	1.16	50.7	69	401	433	3438	500
N602S × LH198	10.10	8.89	1.31	51.2	71	412	437	3662	575
V602S × LH227	11.59	9.14	1.35	56.1	68	420	434	3842	584
V602S × LH244	10.09	9.05	1.24	49.9	70	402	430	3649	535
V602S × LH332	10.26	8.97	1.27	56.0	70	411	439	3717	558
V602S × TR7245	10.24	8.67	1.21	51.7	70	403	439	3493	531
N603S × HC33	8.49	9.31	1.28	50.5	71	395	429	3680	551
V603S × LH198	9.37	9.94	1.56	50.8	71	407	438	4094	654
V603S × LH227	11.16	9.02	1.61	59.7	70	430	446	3907	716
V603S × LH227	8.43	9.02	1.28	51.5	70 71	410	429	3782	550
V603S x LH244 V603S x LH332	10.87	9.24	1.20	48.9	71	410	438	4010	743
	9.11						436		
V603S × TR7245		9.06	1.35	50.2	70	400		3651	593
V604S × HC33	10.13	9.00	1.33	50.2	70	407	439	3687	585
V604S × LH198	10.42	9.35	1.50	47.3	71	408	441	3866	665
W604S × LH227	11.08	8.98	1.48	52.5	70	412	444	3729	656
W604S × LH244	10.60	8.67	1.30	53.1	71	417	444	3627	640
V604S × LH332	12.00	10.18	1.75	50.3	72	418	444	4291	777
N604S × TR7245	10.25	9.03	1.40	48.2	70	404	441	3660	621
V605S × HC33	9.79	9.81	1.21	48.9	70	394	429	3855	522
N605S × LH198	9.85	10.91	1.27	43.5	71	377	430	4132	547
N605S × LH244	12.30	10.11	1.48	49.2	70	394	431	3993	639
N605S × LH332	10.38	9.53	1.44	45.3	71	388	435	3709	627
N605S × TR7245	10.06	10.08	1.24	45.7	71	376	434	3804	540
VQS C3 Syn2	5.14	6.97	0.71	42.6	67	359	419	2526	297
WQS C3 × HC33	8.98	10.11	1.19	50.3	69	386	426	3933	510
VQS C3 × LH198	8.63	9.90	1.23	46.1	71	385	429	3831	529
VQS C3 × LH332	10.05	9.76	1.43	46.5	72	387	431	3788	617
VQS C3 × TR7245	9.28	9.16	1.25	44.8	70	388	433	3580	542
DK5143	11.21	6.97	1.02	55.8	69	426	442	2992	453
HC33 × LH287	11.90	9.47	1.42	54.8	70	403	428	3835	608
_H198 × LH279	12.86	10.69	1.40	51.0	70	390	429	4163	600
.H227 × LH279	12.91	9.25	1.51	59.3	69	424	436	3916	657
H244 × LH287	12.84	9.30	1.45	51.0	70	400	427	3721	620
P34B23	12.43	9.70	1.54	57.4	71	401	437	3851	630
P34M93	12.47	9.97	1.51	58.4	72	422	446	4198	676
P34N44	11.25	8.34	1.08	57.4	70	394	428	3299	288
-697 (bm3)	4.65	7.39	0.67	39.5	72	355	434	2640	469
N48V8 ( <i>Lfy1</i> )	8.33	10.30	1.34	51.2	77	386	426	4014	570
rial mean	9.60	9.11	1.28	49.0	70	394	433	3620	555
rial median	10.24	9.24	1.33	49.9	70	401	434	3782	584
rial range <sup>‡</sup>	8.25	3.94	1.08	20.2	10	75	65	2260	597
.SD (0.05)	2.06	1.30	0.24	6.9	2	26	12	475	101
CV	18.8	13.9	17.2	11.8	1.4	4.6	1.9	14.2	19.6
CV <sub>G</sub> ‡	16.6	9.1	16.2	9.4	2.0	4.0	1.5	9.6	16.5

<sup>&</sup>lt;sup>†</sup>Theoretical ethanol potential (L Mg<sup>-1</sup>) multiplied by stover yield or cob yield.

 $<sup>^{\</sup>ddagger}$ Range and coefficient of genetic variation were calculated excluding the W64A  $\times$  A619 bm isogenic series.

Table 5. Entry means for stover glucan, xylan, and lignin (sum of acid soluble and insoluble) and stover and cob hemicellulose, cellulose, and acid detergent lignin (ADL). Means are over three replications in each of four environments.

				Cob					
Entry	Glucan	Xylan	Lignin	Hemicellulose	Cellulose	ADL	Hemicellulose	Cellulose	ADL
				—g kg <sup>-1</sup> ———				-g kg <sup>-1</sup>	
W64A × A619	288.5	149.6	106.8	308.2	331.1	43.8	406.5	372.0	56.0
W64A × A619 bm1	265.2	132.6	88.8	310.2	323.8	39.3	414.7	338.4	43.8
W64A × A619 bm2	251.7	129.6	88.5	297.1	295.8	33.4	400.2	313.5	43.6
W64A × A619 bm3	265.9	144.5	87.3	317.6	313.8	30.6	414.9	352.6	31.7
W64A × A619 bm4	270.1	142.5	94.9	313.6	320.2	38.8	412.0	357.3	53.7
W601S × HC33	325.4	183.6	114.2	319.1	381.3	47.0	430.7	381.9	67.5
W601S × LH198	315.6	174.3	111.8	313.8	374.7	45.3	432.5	385.5	62.0
W601S × LH227	322.7	183.3	118.5	323.0	381.1	51.9	438.0	383.9	66.3
W601S × LH244	324.7	181.9	113.2	319.0	385.2	49.1	439.6	377.7	62.5
W601S × LH332	325.1	185.8	114.5	325.7	381.4	46.1	436.1	385.0	60.2
W601S × TR7245	326.6	183.6	114.6	319.8	386.4	51.0	432.4	394.9	67.6
W602S × HC33	318.1	181.6	119.5	317.1	377.1	48.3	424.9	378.8	67.7
W602S × LH198	325.7	189.1	120.5	329.6	384.3	47.0	434.1	379.3	60.5
W602S × LH227	329.0	194.4	121.7	332.1	379.9	48.0	431.1	376.7	67.9
W602S × LH244	321.3	183.4	114.7	320.2	376.5	46.3	430.2	370.4	62.1
W6028 × LH332	323.1	187.5	117.9	329.8	377.4	49.4	431.7	383.2	64.1
W602S × TR7245	319.5	183.2	116.0	322.1	377.3	47.6	427.2	386.6	65.2
W603S × HC33	321.6	174.3	119.1	314.1	366.3	46.9	427.9	370.8	62.8
W603S × LH198	327.3	182.1	122.5	324.6	380.3	46.5	434.1	380.0	58.7
W603S × LH227	345.5	195.4	131.4	329.6	402.9	52.2	438.7	389.5	60.7
W603S × LH244	331.0	184.7	121.0	322.3	385.5	46.3	430.5	368.4	60.5
W603S × LH332	330.2	185.0	124.0	330.0	382.0	54.0	431.7	381.8	62.1
W603S × TR7245	324.1	177.1	119.9	318.5	375.7	49.4	428.7	387.6	61.7
W604S × HC33	322.7	181.9	115.1	327.1	370.3	45.6	431.7	383.5	57.6
W604S × H033	323.5	184.0	117.3	328.1	376.3	44.4	440.8	380.2	60.9
W604S x LH227	325.0	187.2	120.3	333.6	370.3	44.4	434.9	388.9	63.7
	334.3		120.3	327.0	384.9	46.1	443.6	383.6	52.9
W604S x LH244		186.2	119.8		380.6			389.3	59.5
W604S x LH332	329.9	189.1		335.4 326.5	375.0	48.8 47.2	435.8 432.8	386.2	59.5
W604S x TR7245	321.7	179.3	115.4	324.2		53.4	432.6		64.0
W605S × HC33	318.1	177.5	124.2		366.4			381.2	
W605S x LH198	302.7 316.9	169.3	118.1 122.4	324.1 328.2	352.9 368.9	48.5 52.7	418.8 420.1	378.7 379.2	59.8 67.7
W605S × LH244		181.0							
W605S × LH332	313.1	173.6	122.1	323.3	364.6	51.7	419.1	387.2	65.0
W605S × TR7245	300.7	171.2	117.6	321.3	351.3	50.8	415.4	388.5	69.4
WQS C3 Syn2	285.5	153.4	96.7	316.7	335.0	36.2	419.0	360.9	52.2
WQS C3 × HC33	307.0	172.0	112.3	324.5	354.2	45.4	422.7	369.0	63.2
WQS C3 × LH198	307.3	172.8	113.2	321.4	362.0	46.8	429.2	369.2	62.7
WQS C3 × LH332	306.0	174.3	113.5	326.7	358.8	47.6	427.9	373.9	63.7
WQS C3 × TR7245	307.6	174.6	113.3	324.6	361.3	47.6	425.6	377.9	60.0
DK5143	340.4	193.7	125.6	325.7	400.6	51.9	434.7	386.2	52.6
HC33 × LH287	323.3	182.3	124.0	331.3	377.3	51.9	419.5	374.5	62.7
LH198 × LH279	308.2	182.1	121.2	329.6	361.3	49.8	428.5	370.6	63.8
LH227 × LH279	335.7	197.1	131.8	340.7	392.7	55.0	427.9	381.6	59.9
LH244 × LH287	321.3	183.5	125.3	327.0	383.1	54.7	425.1	369.5	55.8
P34B23	317.1	185.6	117.6	332.9	374.1	53.6	426.5	383.9	51.1
P34M93	333.2	196.8	124.2	342.0	391.7	53.8	423.4	401.9	59.4
P34N44	315.7	179.9	121.8	322.0	372.9	48.8	426.7	378.9	55.5
F697 (bm3)	281.4	152.9	97.7	311.9	343.7	37.9	421.0	373.8	37.6
N48V8 ( <i>Lfy1</i> )	310.7	173.4	118.5	329.0	360.7	48.1	421.5	370.0	62.4
Trial mean	314.4	176.8	115.7	323.7	368.1	47.4	426.8	376.8	59.4
Trial median	321.3	181.9	118.1	324.5	375.7	47.6	427.9	379.3	60.9
Trial range <sup>†</sup>	64.1	44.2	35.1	30.1	67.8	18.8	30.5	41.0	31.8
LSD (0.05)	18.2	15.7	8.0	12.9	19.5	6.4	10.9	15.0	7.3
CV	4.1	6.4	5.4	3.6	4.4	13.0	1.4	2.5	10.7
CV <sub>G</sub> <sup>†</sup>	6.4	8.8	8.8	2.5	6.0	11.1	2.1	3.8	12.8

 $<sup>^{\</sup>dagger}$ Range and coefficient of genetic variation were calculated excluding the W64A  $\times$  A619 bm isogenic series.

unit mass, which we were unable to measure, compares to TEP. Because actual ethanol production is a function of two variables, TEP and convertibility, there may be more variation for actual ethanol yield than TEP. Differences between entries for theoretical ethanol (TE) yield were large for both plant fractions and were primarily due to differences in yield rather than TEP.

Cob cellulose, hemicellulose, and ADL were greater than their stover counterparts (Table 5). Cob hemicellulose concentration was greater than cob cellulose concentration, and the opposite was observed for the stover samples, which is consistent the greater amount of xylan in cobs (Saha, 2003). Based on the fiber composition results, cobs may contain higher ethanol potential than the vegetative components, but higher ADL concentration (Table 5) will likely make cobs more recalcitrant and expensive to process. It should be noted that preparing cobs for compositional analysis was difficult and time consuming, and the relatively small amount of variation observed may not merit the effort that would be required for breeding.

Stover TEP among the grain hybrids ranged from 390  $L Mg^{-1} (LH198 \times LH279)$  to 426  $L Mg^{-1} (DK5143)$ , a difference that, while significant, was only 6% of the grain hybrid mean. A similar situation was found for cob TEP, where the range among the grain hybrids was only 19 L Mg<sup>-1</sup>. When combined with stover and cob yield, differences in TE yield (L ha<sup>-1</sup>) may be substantial among current hybrids used and bred for grain production. Differences in stover TE yield were primarily driven by differences in stover yield, as can be seen by the performance of DK5143, which had high stover TEP, but low stover TE yield. Stover lignin and cob ADL varied among the grain hybrids, but stover ADL did not (Table 5). However, the ranges in stover lignin and cob ADL concentration were less than 1.4%, and it is not known what impact this small amount of variation will have on convertibility. Dien et al. (2006) found a negative relationship between lignin content and glucose released after dilute sulfuric acid pretreatment, but mature and immature samples of different species were used, and the range in lignin concentration was 6.6%.

# **Evaluation of Germplasm and Mutations Used in Silage Production**

Another objective of our study was to determine if germplasm and mutations used for silage production have characteristics that may make them superior for feedstock production. The top five entries for stover yield consisted of three silage hybrids, the leafy hybrid, and a grain hybrid (Table 4). Four of the five hybrids with the silage inbred parent W605S were statistically indistinguishable from the highest-yielding entry, and all W605S hybrids numerically exceeded the median yield (Table 4). W605S was developed from an Argentine breeding population provided by the USDA Germplasm Enhancement of Maize (GEM) project and is characterized by high forage yield (Nass and Coors, 2003). Bertoia et al. (2002) also found that inbred lines from Argentine populations have more potential for increasing stover yield than inbred lines from the North American Corn Belt. The silage hybrids and grain hybrids are similar with respect to stover and cob TEP: both groups overlap, and both groups are included within one LSD of the best entry for each trait (Table 4). Trends in differences between hybrid types were present for stover lignin, stover ADL, and cob ADL. The silage hybrids tended to have lower stover lignin, with only one grain check among the 24 entries statistically equivalent to the silage hybrid with lowest lignin (Table 5). Inbred lines developed for silage use may be a good source of germplasm for increasing the convertibility of stover. A similar situation was found for stover ADL. However, contrary to expectations, grain hybrids tended to have lower cob ADL than the silage hybrids. For instance, the grain check P34B23 was the fifth lowest in cob ADL and was lower than W64A × A619 bm4 and WQS C3 Syn2 (Table 5). Furthermore, four of the eight grain hybrids were within one LSD of this entry while only two silage hybrids were.

The brown-midrib entries ranked last for stover yield, grain yield, and cob yield (Table 4). With the exception of W64A  $\times$  A619 bm1, the entries isogenic for bm alleles were significantly lower for stover yield than their normal isoline, and all were lower for cob yield. Likewise, the commercial brown-midrib hybrid, F697 (bm3), was not different than the lowest entry for stover yield and cob yield. The brown-midrib entries were susceptible to lodging at physiological maturity, which would result in even poorer performance when machine harvested. The bm1 and bm2 isogenic lines had lower stover and cob TEP than their normal isogenic counterpart but also typically had lower lignin, ADL, and cob ADL, which would reduce recalcitrance and increase convertibility (Dien et al., 2006). The bm3 mutation may hold the most promise for enhancing quality because it significantly decreases all forms of lignin while not significantly reducing stover and cob TEP compared to its normal isogenic counterpart (Tables 4 and 5). The commercial bm3 hybrid also had low lignin and ADL, but TEP was lower as well. Unless yield and agronomic characteristics of brown-midrib hybrids can be improved beyond the levels we observed, their potential for increasing convertibility means little.

The leafy hybrid, N48V8, ranked fourth for stover yield, its cob yield was near average, and its grain yield was below average (Table 4). Isolines of the leafy hybrid were not available, and therefore we do not know if the effects observed were due to genetic background or the *Lfy1* allele per se. Dwyer et al. (1998) showed that leafy hybrids produced more stover than normal hybrids while grain yield was equal, but, as in our study, genetic backgrounds were

different between the leafy and normal hybrids. The presence of extra leaves above the ear conferred by the *Lfy1* allele would be highly advantageous if it could be introgressed into elite grain hybrids without compromising grain yield. The number of leaves conferred by *Lfy1* is, however, dependant on its genetic background (Shaver, 1983). Also, the *Lfy1* allele does increase days to maturity (Table 4; Shaver, 1983); therefore, earliness would need to be bred into leafy hybrids to be acceptable for grain production.

The WQS C3 Syn2 population was evaluated on a per se basis and also in hybrid combination with four commercial testers. The compositional properties of the WQS C3 Syn2 population were similar to those of the brown-midrib entries (Table 5). One exception was cob ADL, which was higher in WQS C3 Syn2 than the brown-midrib entries, while stover ADL was not different between these entries. Selection for silage digestibility apparently has not impacted cob ADL as much as stover ADL.

The average stover yield of the testcrosses was 40% higher than the WQS C3 Syn2 per se stover yield. The average grain and cob yields of the testcrosses were both 80% higher than the per se performance of the synthetic population, indicating that while hybridization significantly increased stover yield, the relative increase was not as

large as that observed for the ear traits. This finding agrees with prior evaluations of stover and ear yield heterosis at silage harvest (Moreno-Gonzalez et al., 2000). Although the WQS population has undergone recurrent selection for testcross performance of silage yield and quality, testers are still chosen based on the common Stiff Stalk—Non Stiff Stalk heterotic pattern. Current heterotic patterns have been created through selection primarily on grain yield (Duvick et al., 2004). Alternative heterotic patterns that increase stover yield heterosis should be sought and could be a major, immediate source of increased stover yield.

## **Combining Ability Analysis**

A balanced subset of the silage hybrids was used for a combining ability analysis to determine the importance of variation due to GCA and SCA effects. Differences were found for all traits among the 25 hybrids. Silage inbred GCA effects were significant for several yield and composition traits, including stover yield, cob yield, stover TEP, and cob TEP (Table 6). W605S was the only silage inbred with a significantly positive GCA effect for stover yield, but it also had significantly lower TEP (Table 7). Because of the exotic pedigree of W605S, it may be assumed that high stover yield and low polysaccharide concentrations

Table 6. Significance of general (GCA) and specific combining ability (SCA) effects and their interactions with environments in factorial mating design involving five maize silage inbreds and five tester inbreds. The number of significant crossover interactions (COIs) for silage inbred and tester inbred GCA effects over environments was calculated for traits where the GCA × environment (E) source of variation was significant.

	Silage inbred	Tester	004	Silage inbred	Tester inbred	SCA	SS	No. signific	cant COIs‡
	GCA	inbred GCA	SCA	GCA × E	GCA × E	×Ε	ratio†	Silage inbred	Tester inbred
df	4	4	16	12	12	48			
Grain yield	NS§	NS	NS	*	NS	NS	0.57	7	_
Stover yield	*	NS	*	**	NS	NS	0.71	6	_
Cob yield	*	**	**	**	*	NS	0.73	2	4
Stover DM <sup>¶</sup>	**	NS	NS	**	NS	NS	0.76	3	_
Stover TEP¶	**	NS	NS	*	NS	NS	0.78	4	_
Cob TEP¶	*	**	NS	**	NS	NS	0.87	2	_
Stover TE yield¶	NS	*	NS	**	NS	NS	0.66	5	_
Cob TE yield <sup>¶</sup>	*	**	*	**	NS	NS	0.77	2	_
Stover glucan	**	NS	NS	*	*	NS	0.76	3	7
Stover xylan	*	NS	NS	NS	NS	NS	0.66	_	_
Stover lignin	**	NS	NS	NS	**	NS	0.73	_	10
Stover hemicellulose	NS	NS	NS	*	NS	NS	0.67	5	_
Stover cellulose	**	NS	NS	NS	*	NS	0.76	_	7
Stover ADL¶	**	NS	NS	NS	NS	NS	0.65	_	_
Cob hemicellulose	**	**	NS	**	NS	NS	0.95	12	_
Cob cellulose	NS	**	*	**	NS	NS	0.80	7	_
Cob ADL¶	NS	**	*	**	NS	NS	0.66	11	_

<sup>\*</sup>Significant at 0.05 probability level.

<sup>\*\*</sup>Significant at 0.01 probability level.

<sup>†</sup>Ratio of (inbred sums of squares + tester sums of squares) to hybrid sums of squares (Pixley and Frey, 1991).

<sup>‡</sup>Out of 60 possible tests

<sup>§</sup>NS, not significant.

Stover DM, stover dry matter; TEP, theoretical ethanol potential; TE yield, TEP multiplied by stover or cob yield; ADL, acid detergent lignin.

were related to longer maturity time; however, stover DM and days to midpollen values were average for W605S (Tables 4 and 7). W604S had superior GCA for both TEP and ADL (Table 7), making it a potentially good source of germplasm for both ethanol potential and convertibility. Tester GCA effects were significant for cob traits and stover TE yield (Table 6). For instance, hybrids with LH332 as one parent have the potential to produce 146 L ha<sup>-1</sup> more ethanol than hybrids with parent TR7245 (Table 7). Also, the GCA effect of LH332 for cob yield was 181 kg ha<sup>-1</sup>. SCA effects were significant for stover, cob, and cob TE yield as well as for cob cellulose and ADL (Table 6). The silage inbred + tester GCA sums of squares to hybrid sums of squares ratio (SS ratio; Pixley and Frey, 1991) was used to estimate the relative importance of additive and nonadditive genetic effects. The SS ratio did not show a clear pattern between trait types. As expected, the SS ratio was lowest for grain yield. Stover and cob yield were both around 0.71, and the composition traits ranged from 0.65 to 0.95 (Table 6).

General combining ability × environment interaction effects were significant for 15 of the 17 traits measured. We studied the GCA × environment interaction of these traits further by testing for significant COIs between each pair of silage inbreds or tester inbreds in each pair of environments (Table 6). Of the 60 possible COIs for each trait, significant COIs ranged from two to 12 across all traits. Grain yield and stover yield had a similar number of significant COIs among the silage inbreds. The presence of significant COIs for stover and cob composition suggests that

multienvironment evaluation, similar to what is needed for yield traits, will also be required for accurate selection of stover and cob quality. For instance, there were 10 significant COIs among the tester inbreds for stover lignin. Significant GCA × environment effects are commonly found for stover digestibility measurements (Dhillon et al., 1990; Argillier et al., 2000), further suggesting the complexity of stover composition and convertibility.

### **Relationships between Traits**

There were a large number of significant correlations between traits (Tables 8-10). For the 29 silage hybrids, the correlation between grain yield and stover yield, while positive in direction, was not significantly different than zero. Hybrids can be identified that have both high stover yield and high grain yield (Fig. 1A). The correlation between grain yield and cob yield was positive but only moderate in magnitude (r = 0.56; Fig. 1B). While there were differences in shelling percentage among the 29 silage hybrids as well as among inbred and tester GCA effects (data not shown), the range in shelling percentage among the silage hybrids only ranged from 83.8 to 87.7%. Few unfavorable correlations were found among these traits. There was a negative correlation between stover yield and stover DM and a small negative correlation between stover yield and stover TEP. Stover DM is related to days to flowering (r = -0.38; data not shown) and grain moisture (Wolf et al., 1993), and maintaining acceptable maturity and grain moisture could be more challenging when breeding for both grain and stover yield compared

Table 7. General combining ability (GCA) effects for maize silage inbreds and tester inbreds.

		Sil	age inbre	ds			Te	ster inbre	ds		Mean
	W601S	W602S	W603S	W604S	W605S	HC33	LH198	LH244	LH332	TR7245	
Grain yield, Mg ha <sup>-1</sup>	0.50	-0.14	-1.01	0.42	0.22	-0.32	-0.24	0.20	0.65	0.31	10.3
Stover yield, Mg ha-1	0.41	-0.71*	-0.06	-0.25	0.59*	-0.22	0.27	-0.03	0.25	-0.31	9.59
Cob yield, Mg ha <sup>-1</sup>	0.09	-0.15**	0.04	0.07	-0.06	-0.10*	0.03	-0.03	0.18**	-0.09*	1.39
Stover DM <sup>†</sup> , %	-3.46**	3.10**	1.64	1.03	-2.33	0.50	-1.79*	1.12	0.50	-0.35	48.6
Stover TEP†, L Mg <sup>-1</sup>	2.91	3.22	2.28	8.38*	-16.81**	-1.44	-3.00	3.50	5.13	-4.21	402
Cob TEP <sup>†</sup> , L Mg <sup>-1</sup>	3.98*	-1.52	-2.21	4.99*	-5.25**	-3.60**	0.34	-2.27*	2.55*	2.97**	437
Stover TE yield,† L ha-1	92.29	-114.90	0.95	-6.05	27.73	-46.30	39.99	6.01	73.33*	-73.01*	3840
Cob TE yield,† L ha <sup>-1</sup>	37.46	-67.87*	11.39	50.70	-31.66	-45.18**	0.84	-3.13	83.31**	-35.83*	613
Stover glucan, g kg <sup>-1</sup>	1.78	-0.19	5.15	4.70	-11.42**	-0.51	-2.74	3.91	2.54	-3.20	322
Stover xylan, g kg <sup>-1</sup>	0.61	3.71*	-0.50	2.87	-6.72**	-1.39	-1.45	2.19	2.97	-2.34	181
Stover lignin, g kg <sup>-1</sup>	-4.59**	-0.42	3.05**	-0.69	2.64	0.25	-0.20	0.00	1.46	-1.53	118
Stover hemicellulose, g kg <sup>-1</sup>	-4.16	0.10	-1.71	5.18	0.61	-3.29	0.40	-0.26	5.14	-1.99	323
Stover cellulose, g kg <sup>-1</sup>	6.49*	3.26	2.67	2.11	-14.52**	-2.95	-1.62	4.87	1.88	-2.19	375
Stover ADL,† g kg-1	-0.65	-0.62	0.22	-1.95**	3.02**	-0.13	-2.02	-0.28	1.64	0.80	48.4
Cob hemicellulose, g kg <sup>-1</sup>	4.50	-0.17	0.88	7.21**	-12.43**	-4.10**	2.32*	3.07**	1.10	-2.41**	430
Cob cellulose, g kg <sup>-1</sup>	3.04	-2.31	-4.29	2.58	0.98	-2.73*	-1.24	-6.10**	3.35**	6.71**	382
Cob ADL,† g kg <sup>-1</sup>	1.48	1.47	-1.24	-4.43	2.71	1.48	-2.10**	-1.35	-0.18	2.14**	62.2

<sup>\*</sup>Significant at 0.05 probability level.

<sup>\*\*</sup>Significant at 0.01 probability level.

<sup>†</sup>Stover DM, stover dry matter; TEP, theoretical ethanol potential; TE yield, TEP multiplied by stover or cob yield; ADL, acid detergent lignin.

to breeding for grain yield alone. One very important observation was the much stronger relationship between TE yield and stover yield compared with that between TE yield and TEP (Fig. 2). Because TE yield is a function of TEP and stover yield, this result simply reflects the larger amount of genetic variation for stover yield than for stover composition. A similar result was found for cob TE yield.

The high correlation between cellulose and TEP (r = 0.91; Table 8) followed from the high correlations between cellulose, measured by the detergent fiber system, and both glucan and xylan predicted with the Stover9 NIRS calibration. If advances in pretreatment and enzyme hydrolysis make TEP the most important variable in stover quality, the simple and rapid detergent analysis may

be adequate for predicting TEP for breeding purposes. Jung and Lamb (2004) also reported a high correlation (r = 0.96) between detergent cellulose and glucose analyzed by the Uppsala dietary fiber method in alfalfa stem samples obtained from different genotypes and harvest times. Also, the results of Jung and Lamb (2004) were

similar to our results in that correlations between detergent fiber system-Uppsala dietary fiber measurements were significant, but relatively low for the hemicellulose and lignin fractions. Furthermore, independent predictions of Uppsala fiber fractions using regression equations based on the detergent fiber system data were unsuccessful for hemicellulose and lignin, but deviations were small for cellulose. Additional evidence for the limitations of using detergent fiber data to quantify cell wall concentration comes from smooth bromegrass, where successful divergent selection on NDF concentration did not result in plants with altered total cell wall concentrations or composition, but rather altered cell wall solubility in ND solution

Table 8. Phenotypic correlations (n = 29) between grain yield, stover yield, cob yield, stover dry matter (DM), stover theoretical ethanol potential (TEP), cob TEP, stover TE yield (stover TEP multiplied by stover yield), and cob TE yield.

	Grain yield	Stover yield	Cob yield	Stover DM	Stover TEP	Cob TEP	Stover TE yield
Stover yield	0.22						
Cob yield	0.56**	0.41*					
Stover DM	0.08	-0.60**	-0.04				
Stover TEP	0.34	-0.44*	0.42*	0.69**			
Cob TEP	0.37*	-0.24	0.52**	0.19	0.65**		
Stover TE yield	0.43*	0.87**	0.71**	-0.31	0.06	0.12	
Cob TE yield	0.60**	0.31	0.97**	0.04	0.51**	0.62**	0.65**

<sup>\*</sup>Significant at 0.05 probability level.

(Casler and Hatfield, 2006). Therefore, use of the detergent fiber analysis for quantifying biofeedstock quality may depend on species and which cell wall fractions (i.e., total cell wall or cellulose) are targeted for improvement.

There was a small correlation between stover and cob ADL, but there was no stover—cob relationship for cellulose

Table 9. Phenotypic correlations (n = 29) between yield traits and composition traits.

	Grain yield	Stover yield	Cob yield	Stover DM†	Stover TEP <sup>†</sup>	Cob TEP <sup>†</sup>	Stover TE yield <sup>†</sup>	Cob TE yield <sup>†</sup>
Stover glucan	0.19	-0.40*	0.42*	0.66**	0.95**	0.57**	0.07	0.51**
Stover xylan	0.46*	-0.40*	0.35	0.74**	0.94**	0.51**	0.06	0.43*
Stover lignin	0.06	-0.12	0.18	0.61**	0.28	-0.09	-0.01	0.20
Stover hemicellulose	0.56**	-0.02	0.43*	0.49**	0.51**	0.32	0.24	0.50**
Stover cellulose	0.23	-0.40*	0.39*	0.53**	0.91**	0.64**	0.05	0.45*
Stover ADL <sup>†</sup>	0.19	0.18	0.09	0.04	-0.15	-0.18	0.09	0.08
Cob hemicellulose	0.20	-0.30	0.47*	0.31	0.78**	0.80**	0.12	0.56**
Cob cellulose	0.35	-0.02	0.27	-0.07	0.09	0.66**	0.05	0.33
Cob ADL <sup>†</sup>	0.18	0.01	-0.23	-0.12	-0.30	-0.32	-0.15	-0.32

<sup>\*</sup>Significant at 0.05 probability level.

Table 10. Phenotypic correlations (n = 29) between composition traits.

	Stover glucan	Stover xylan	Stover lignin	Stover hemicellulose	Stover cellulose	Stover ADL <sup>†</sup>	Cob hemicellulose	Cob cellulose
Stover xylan	0.83*							
Stover lignin	0.39*	0.33						
Stover hemicellulose	0.37*	0.63**	0.46*					
Stover cellulose	0.92**	0.82**	0.22	0.23				
Stover ADL <sup>†</sup>	-0.06	-0.08	0.50**	0.06	-0.06			
Cob hemicellulose	0.73**	0.64**	-0.13	0.25	0.76**	-0.43*		
Cob cellulose	0.03	0.05	0.00	0.22	0.10	0.25	0.08	
Cob ADL <sup>†</sup>	-0.38*	-0.12	-0.05	-0.24	-0.23	0.43*	-0.50**	0.09

<sup>\*</sup>Significant at 0.05 probability level.

<sup>\*\*</sup>Significant at 0.01 probability level.

<sup>\*\*</sup>Significant at 0.01 probability level.

<sup>†</sup>Stover DM, stover dry matter; TEP, theoretical ethanol potential; TE yield, TEP multiplied by stover or cob yield; ADL, acid detergent lignin.

<sup>\*\*</sup>Significant at 0.01 probability level.

<sup>†</sup>ADL, acid detergent lignin.

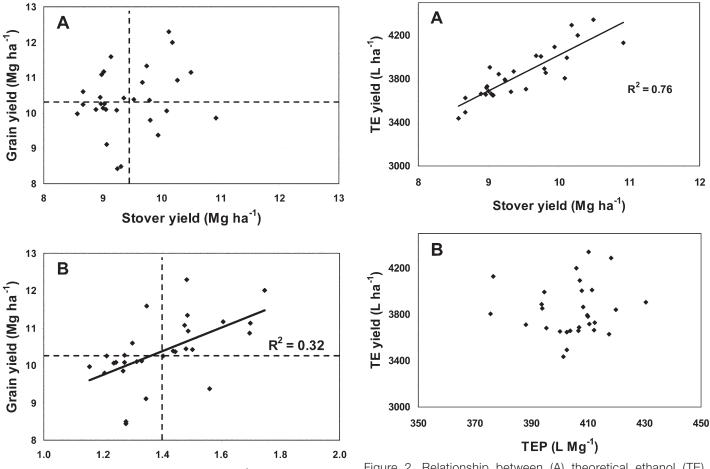


Figure 1. Relationship between maize grain yield and stover yield (A) and grain yield and cob yield (B). Points are means of silage hybrids (n = 29) over four environments. Reference lines indicate grand mean for each trait.

Cob yield (Mg ha<sup>-1</sup>)

and hemicellulose (Table 10). Stover cellulose was correlated to cob hemicellulose (r = 0.76). Because of the aforementioned limitations of the Van Soest detergent system in measuring cell wall composition based on dry matter solubility (Jung, 1997; Theander and Westerlund, 1993), we do not know if there are genetic mechanisms underlying this observation or if it is a method artifact. For instance, the ADF fraction is often contaminated with hemicellulose residues (Morrison, 1980), and cob hemicellulose may be more readily solubilized in AD solution than stover hemicellulose. In this case, variation in stover hemicellulose, which would lead to the high correlation we observed between stover cellulose and cob hemicellulose.

The IVR assay results reinforce the notion that there are substantial differences in ruminal digestibility of stover between maize hybrids, and that digestibility is enhanced by reducing lignin content of the stover (Fig. 3). The brownmidrib hybrids were actually lowest for TEP but were highest for IVR gas production, in accord with the known negative effects of lignin on forage digestibility (Jung and

Figure 2. Relationship between (A) theoretical ethanol (TE) yield and stover yield and (B) TE yield and theoretical ethanol potential (TEP). Points are means of silage hybrids (n = 29) over four environments.

Deetz, 1993). There was no relationship between stover TEP and IVR gas production of the non brown-midrib hybrids (Fig. 3). This disparity is undoubtedly due in part to the use in the latter assay of ground stover samples that had not been subjected to additional physical or chemical pretreatments. The IVR assay was originally developed as a facile means of screening biomass materials for their suitability for conversion by consolidated bioprocessing (CBP), a bioconversion platform that combines enzyme production, enzymatic hydrolysis, and fermentation of the resulting sugars in a single bioreactor. The IVR assay is based on a mixed microbial population that possesses a variety of enzymes capable of hydrolyzing accessible polysaccharides, and fermenting the resulting sugars (both hexoses and pentoses), a configuration similar to a mature CBP platform (Lynd et al., 2008) The IVR assay also mimics two desired, yet currently unattained, goals of SSF (effective enzyme hydrolysis and a hexose/pentose cofermentation) that underlie the TEP calculation. It has been demonstrated that the IVR assay is well correlated with a bench-scale SSF assay when using several different species of non-pretreated grasses (Weimer et al., 2005). In that study, the range in both IVR gas production and ethanol production by SSF

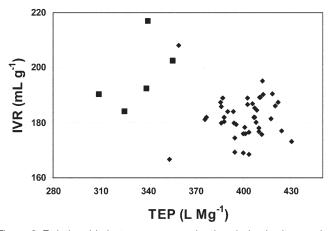


Figure 3. Relationship between gas production during in vitro ruminal fermentation (IVR) and theoretical ethanol potential (TEP). Points (n=49) represent means over replications and locations in 2005 (brown-midrib entries =  $\blacksquare$  and non brown-midrib entries =  $\spadesuit$ )

varied substantially within each forage type tested (eastern gamagrass, big bluestem, and switchgrass). By contrast, in the present study, variations in both IVR and TEP for non brown-midrib maize stover were small (Fig. 3). The fact that both estimates of biomass fermentability show only small ranges among the sample population suggests that the properties of the biomass materials will have relatively little impact on total ethanol production. However, direct comparisons between TEP and either ethanol production via SSF or gas production by the IVR assay, will require the use of substrates that have been pretreated to maximize the fermentability of biomass

In summary, we found large differences between hybrids and GCA effects for maize traits that could impact the economics of cellulosic ethanol production. Before dedicated breeding efforts begin, large increases in the amount of ethanol produced from stover on an area of land might be accomplished through hybrid screening and recommendation. A large screening of elite grain hybrids should be undertaken to study this potential impact further. Germplasm and mutations used in breeding maize for silage production may be useful material for increasing stover yield and reducing lignin. There were significant GCA effects for nearly all traits, and SCA effects were significant for stover yield, cob yield, and cob TEP. We found significant COIs for nearly all traits, suggesting that multienvironment evaluation will be needed for improving both yield and quality of maize stover. Stover yield and grain yield were independent. The common Stiff Stalk-Non Stiff Stalk heterotic pattern used in breeding for grain yield may not be optimal for stover yield; alternative heterotic patterns that increase heterosis for stover yield should be sought. We found more variability for stover yield than TEP, which indicates that the shortest route to increasing ethanol yield on an area basis will be through breeding for stover yield. If TEP becomes the most salient

variable in feedstock quality, the easy and rapid detergent fiber analysis could be used for ranking breeding material in lieu of more advanced and costly methods. However, as suggested by the lack of relationship between IVR and TEP, more research is needed to relate TEP, compositional properties, and conversion efficiency to an overall measure of feedstock quality.

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